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Histological study of development liver in Indigenous Rabbits Fetuses

Bassim Abdullah Jassim
College of science
Al-muthana university

Jaffar Ghazi Abbas al- jebori
College of veterinary med.
Al-Qasim green university

Ahmed Mahdi Saleh
College of science
Wasit university

Abstract

Twenty mature indigenous breed rabbits were used, the animals were divided into four groups(A, B, C, D, and E) each group composed of four female and one male according to times of embryonic study which included (6, 8, 10, 12, 14) days postnatal. This study showing the histological changes during the development of liver in early embryonic life by using the H&E Stain. The results at six day of gestation differentiation of some structures as a clusters of stem cells in deferent location of rabbit embryo. The cluster of stem cells will development into deferent organs in the next stage of development of rabbit embryo. The result at eight day of gestation appear the liver as spherical structure near the heart development region in addition to the appear of the other organs beside the fetal liver. At ten day of gestation the embryonic liver become more prominent and differentiation inside the embryo. The twelve day of embryonic life the proliferation of hepatoblast and showing in deferent figure of mitotic division. The result appear the primordial hepatoblast arranged as short hepatic cords and located some of small lipid droplets in cytoplasm of hepatoblast. At fourteen day of gestation the hepatocytes arranged as long hepatic cords, the lipid droplets disappear from the cytoplasm in addition to located high number of hematopoietic cells have nucleus in blood stream, so the density of outer region of foetal liver more than the central region.

Introduction

The liver is the largest internal organ providing essential metabolic, have exocrine and endocrine functions. These include production of bile, metabolism of dietary compounds, detoxification, regulation of glucose levels through glycogen storage and control of blood homeostasis by secretion of clotting factors and serum proteins such as Albumin, [1].

Hepatocytes are the principal cell type in the liver accounting for ~70% of the mass of the adult organ. Hepatocytes, along with biliary epithelial cells (BECs; also known as cholangyocytes) are derived from the embryonic endoderm, while the stromal cells, stellate cells, kuppfer cells and blood vessels, are of mesodermal origin. The hepatogenesis is evolutionarily conserved and occurs through a progressive series of reciprocal tissue interactions between the embryonic endoderms and nearby mesoderm [2].

The application of this information has recently enabled researchers to produce “hepatic-like” tissue from embryonic stem (ES) cells in vitro, which may ultimately lead to therapeutically useful tissue for transplantation [3].

Shortly after hepatic specification the epithelium begins to express liver genes (*Albumin*, *Afp*,*Hnf4α*) and thickens as the cells transition from a simple cuboidal to a pseudostratified columnar epithelium, thus forming the liver diverticulum [4].

The liver bud undergoes tremendous growth and becomes the major site of fetal haematopoiesis. This growth is regulated by paracrine signals from hepatic mesenchyme, as well as by genes that act intrinsically in the hepatoblasts. Mutations in many of these genes result in a similar embryonic lethality due to impaired hepatoblast proliferation and/or increased cell death, which often causes severe anemia because the defective liver cannot support fetal haematopoiesis [5].

The differentiation of hepatoblasts into hepatocytes begins around of mouse development. Initially hepatoblasts express

genes associated with both adult hepatocytes as well as fetal liver genes such as α-fetoprotein (*Afp)*. Hepatoblasts in contact with the portal vein form a monolayer, and then a bi-layer [6].

The perinatal period focal dilations appear in the bi-layer and these become surrounded by portal mesenchyme, while the remaining bi-layer cells regress. This process, which involves tubulogenesis and apoptosis, is known as ductal plate remodeling. [7, 8].

The hepatoblasts in the liver parenchyma that are not in contact with portal veins gradually differentiate into mature hepatocytes [9].

The hepatocytes acquire their characteristic epithelial morphology arranged in hepatic chords with bile canaliculi on the apical surfaces. While defects in early liver bud growth are often embryonic lethal, disruption in hepatocyte maturation/function or ductal plate remodeling malformations are observed in many human [6].

The lumen of both the intrahepatic and extrahepatic biliary tract they have distinct developmental origins with the gall bladder and extrahepatic bile ducts (EHBD) arising from the caudal portion of the hepatic diverticulum [10].

During emberunic development, the stem cells of the blastocyst from the inner cell mass will differentiate into tissue-specific progenitor cells Fate-mapping experiments have shown that the liver arises from the lateral domains of endoderm in the ventral foregut and from a small group of cells tracking down the ventral midline[11].

During foregut closure, the medial and lateral domains fuse together and the endoderm cells are specified to a hepatic fate under the influence of inductive signals and genetic regulatory factors that are highly conserved among vertebrates [12].

Following hepatic specification of the foregut endoderm, the cellular responses to inductive signals elicit new gene expression programmes required forcell differentiation. When signalling, initially repressed by inhibitors to maintain foregut identity and allow hepatic induction becomes necessary to promote liver bud emergence and differentiation [13].

The newly specified hepatic cells, at this stage referred to as hepatoblasts, change to a columnar shape and invade the septumtransversum mesenchyme to form the liver bud. This transition involves coordinated interkinetic nuclear migration andproliferation, loss of intercellular adhesion, hepatoblast migration [14].

Once hepatoblasts bud into the local mesenchyme, they continue toproliferate under the influence of a variety of cytokines and growth.factors secreted by mesenchymal cells in the septum transversum Stimulatory signals to hepatoblasts from neighbouring endothelial cellsare of particular importance as the presence of endothelial cells ، independent of the blood supply, is critical for normal liver organogenesis throughout development [15].

3.Materials and Methods

3.1. Animal mating and husbandry

The research was applied on the local domestic rabbits (*Oryctolagus Cuniculus*), in al- samawa city. Mature female rabbits weighing (2-2.5) k.g and age (1.2- 1.6) years. The work done in the animal house of Biology Department, Collage of science / AL- Muthana University.

3.2. Experimental design and surgical procedure

The study was performed on twenty five mature indigenous rabbit´s. The experimental animals were divided into five groups each groups composed of four female and one male. The animal groups which named as (A, B, C, D, and E)distributed among the study times (6, 8, 10, 12, 14) days postnatal.

The pregnant female of each group was scarified by using the chloroform, obtain on the embryo by surgical incision of the abdominal wall after that reached to the uterine horns which contain on the embryos'. The embryos were exited from the horns of uterus by opening the wall of the horns and were put its in container with normal saline.

Histological study

The samples of fetal livers taken after exited of embryo from the uterin horn. The samples were washing by normal saline after that the samples will pass through the following steps:

1-fixation by formaldehyde(10%) for 48

hours. [16].

2- Dehydration

3-Clearing : [17].

4-Embedding : (16, 18).

5-Blocking.

6-Cutting: [19.]

7-Staining:

The sections are stained with (Alum Haematoxylin and Eosin).

Results

The early development stages of rabbit embryo which beginning to differentiation of some structures as a clusters of stem cells in deferent location of rabbit embryo. The cluster of stem cells will development into deferent organs in the next stage of development of rabbit embryo. The early differentiation structures which include the Hepatic premordium, foregut, septum transversum, coelomic cavity, neural tube

Embryonic liver at eight day of gestation:

The rabbit embryo at this age characterized by differentiation some of organs inside of the body of embryo , this organs which include the heart, gut tubes, lungs buds, and liver. The liver at this age characterized by the aggregation of high numbers of stem cells in early stages with hipper chromatic nuclei appear pale in colour and figure(1). The histological section of fetal liver of rabbit embryo in fig.(2) showing the location of liver near the septum transversum caudal and ventral to the developed heart.

The foetal liver was contained by the great numbers of hematopoietic cells. The cytoblasem of hepatocyte have some of lipid droplets small in size. The hepatocyte have large pale spherical nucleus. The bile duct and hepatic artery Fetal liver at 14 day of gestation:

The hepatocytes more proliferation than the other preveous stages of development and more differentiation. The cytoplasm of hepatocyte don't have lipids droplets fig.(5). The density of liver was increased and to formation of the liver lobes. The kupffer cells was more prominent than the previous stage of development. The The result at six day of gestation the result showing the rabbit embryo at this age characterized by differentiation some of organs inside of the body of embryo , this organs which include the heart, gut tubes, lungs buds, and liver. The liver at this age characterized by the aggregation of high numbers of stem cells in early stages this result similar to the result of

Embryonic liver at six day of gestation:

Spotted in shape and differentiation into two light and dark regions.

Embryonic liver at ten day of gestation:

At this time the primordial hepatic cells were observed in several shapes of mitotic figures, and staring to increased in number. The proliferation of the primordial cells in deferent location as a clusters in the area of liver development. The primordial cells will more proliferation and differentiation to hepatoblaste. The hepatoblast inter several mitotic division with large nucleus to form the essential amount of hepatocytes in deferent stage of mitosis division. The cytoplasm of hepatoblaste have numerous of lipid droplets. The portal area become more dilation, hepatic cords appear more prominent and arranged as arrows around the portal area. The proliferation of the hepatocytes more than the previous stage of development of foetal liver fig. (3). The sinusoids appear as narrow spaces between the cords of hepatocytes.

Fetal liver at twelve day of gestation:

embedded in the liver mesenchyma. The connective tissue capsule covering the external surface of liver the outer layer composed of a single layer of mesothelial cells fig.(4). The kupffer cells have irregular nucleus.

hematopoietic activity has elevated and the erythroblast accumulation inside of the blood stream in the foetal liver. The density of hepatocytes in outer region of liver more than the central region.

Discussion:

(20) which referred to The inner body cavities filled with organs, and organs come from the innerendoderm. These include lungs and respiratory system, liver, digestive system and intestinal tract and endocrine glands

The results of [21] which referred to during embryonic development, stem cells of the blastocyst inner cell mass differentiate into

multipotent tissue-specific progenitor cells Fate-mapping experiments have shown that the liver arises from the lateral domains of endoderm in the ventral foregut and from a small group of cells tracking down the ventral midline in mammals.

The transverse_septum differentiates to form the hepatic diverticulum and the hepatic primordium, these two structures together will go on to form different components of the mature liver and gall bladder. The rapidly developing liver also forms a visible surface bulge on the embryo directly under the heart bulge. The liver now occupies the entire ventral body cavity with parts of the gastrointestinal tract [22].

The result at the eight day of gestation the primordial hepatic cells were observed in several mitotic figures, and staring to increased in number. The proliferation of the primordial cells in deferent location as a clusters in the area of liver development, that agreed with the results of (7,8) are found that The differentiation of hepatoblasts into hepatocytes begins around of mouse development. Initially hepatoblasts express genes associated with both adult hepatocytes. Hepatoblasts in contact with the portal vein form a monolayer. The perinatal period focal dilations appear in the bi-layer and these become surrounded by portal mesenchyme, while the remaining bi-layer cells regress. This process, which involves tubulogenesis and apoptosis, is known as ductal plate remodeling.

The oval cells are thought to be the progeny of a liver stem cell compartment and strong evidence now exists indicating that these cells can participate in liver regeneration by differentiating into different hepatic lineages [23].

The result at ten day of gestation which showing the fetal liver was contained the great numbers of hematopoietic cells. The cytoblasem of hepatocyte have lipid droplets small and moderate in size Similar to the result which referred to thefoetal liver of mice, this result confirmed to the finding of [24], which showing that the foetal liver of mice in early stage of development, the lipid droplets small and moderate in size could be seen easy in cytoplasm of hepatocyte but they were not as abundant as lipid in liver from neonatal and adult animals.

The result of fetal liver at twelve day of gestation which The hepatocytes more proliferation than the other previous stages of development and more differentiation similar to the results of [8, 25] were found the hepatocytes acquire their characteristic epithelial morphology arranged in hepatic chords with bile canaliculi on the apical surfaces. While defects in early liver bud growth are often embryonic lethal, disruption in hepatocyte maturation and function or ductal plate remodeling malformations are observed in many mammalian disorders.

The three phases of liver development that are the focus of this review are: the specification of hepatoblasts within the endoderm, the lineage split of hepatoblasts into hepatocytes and biliary cells, and the interaction of these cells with different mesodermal cell derivatives during liver morphogenesis[20].

The result of foetal liver at fourteen day of gestation also similar to the finding of [26, 27] which decided that the hepatocytes differentiation into specific fully functional adult cell lineages remains a significant challenge. The use of foetal liver progenitors cells have undergone sufficient morphological and physiological differentiation so that they are committed to hepatic fate, maintaining their bipotentiality and proliferative capacity.

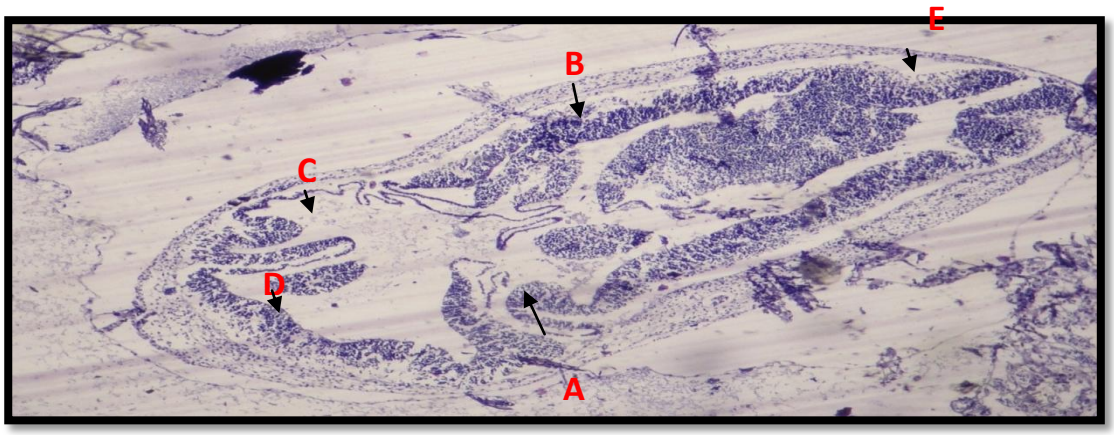

Fig.(1) embryo at 6 day of gestation shows the deferent structures of embryo which include A-hepatic premordium. B-forgut. C- septum transversum. D. coelomic cavity. E-neural tube. H&E.stain. (100X).

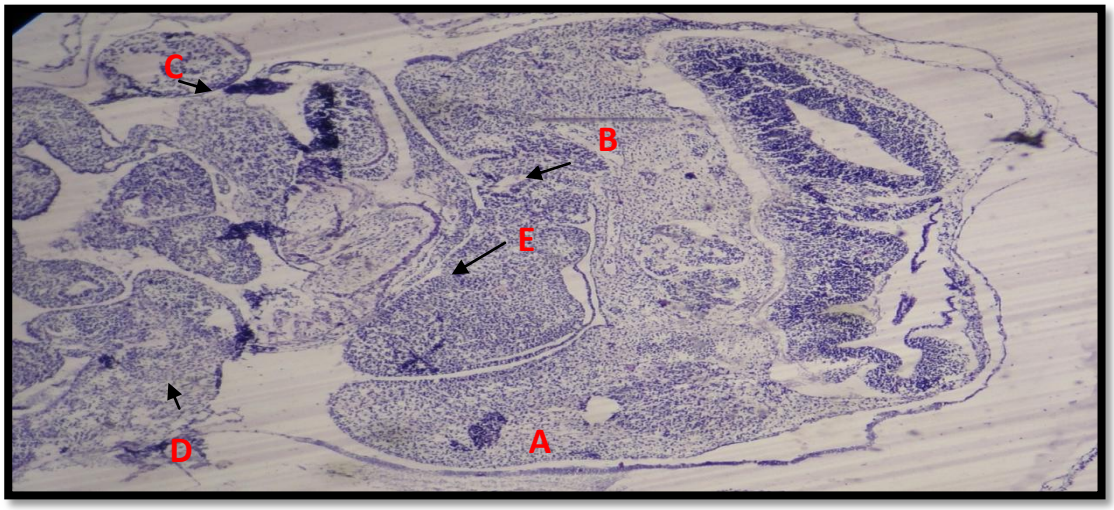

Fig (2) Embryo at 8 day of gestation that shows the following, A- Hepatic primordial. B- Hepatic premordium. C- left dorsal aorta. D- Right dorsal aorta. E- Midgut. H&E stain (100X).

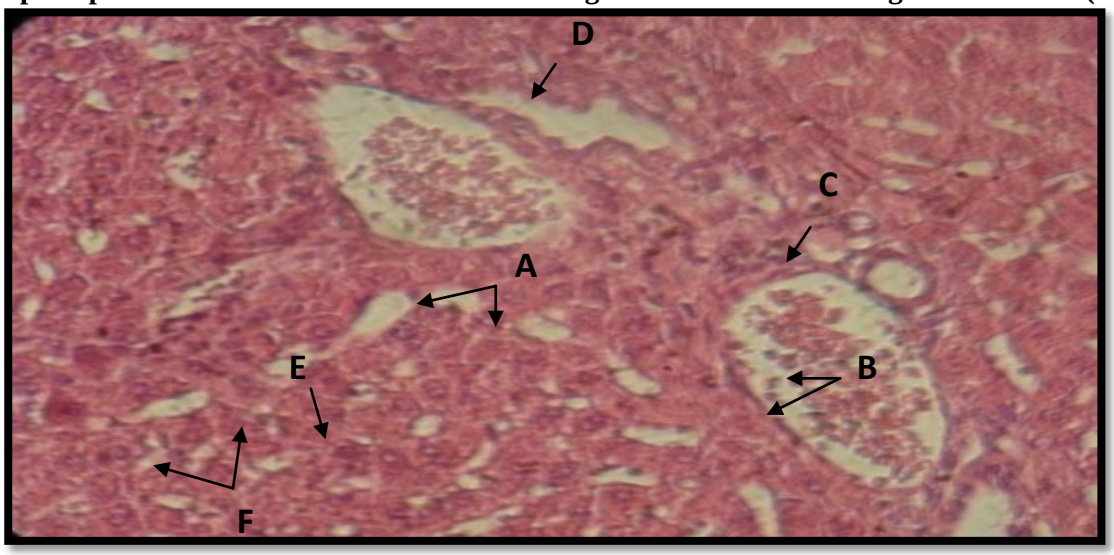

Fig.(3): fetal liver at 10 day of gestation which shows the following A- hepatoblast in deferent mitotic figures with spherical nucleus. B-heamatopoietic cells in early stages with nucleus in the blood vessels of fetal liver.C-Early portal area. D-Primary hepatic sinusoid. E-Biliary ducts. F- Lipid droplets. H&E Stain (100x).

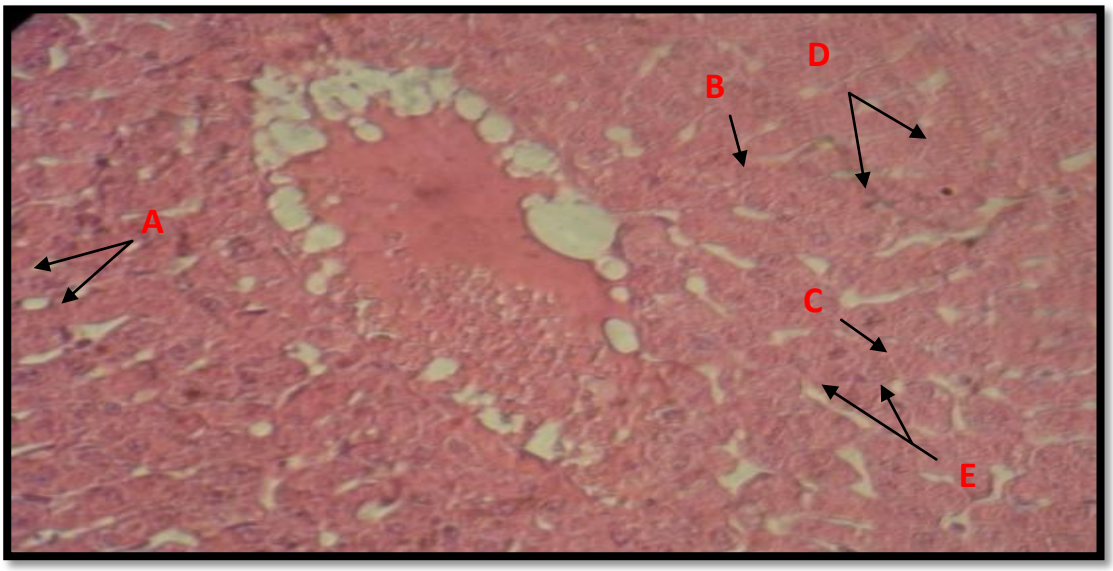

Fig. (4): fetal liver at 12 day of gestation which shows the A- Primary hepatic cords. B- Hepatocyte in mitotic divisions with large nucleus. C- Kupffer cells. D- Liver sinusoids. E- lipid droplets. H&E Stain (100x).

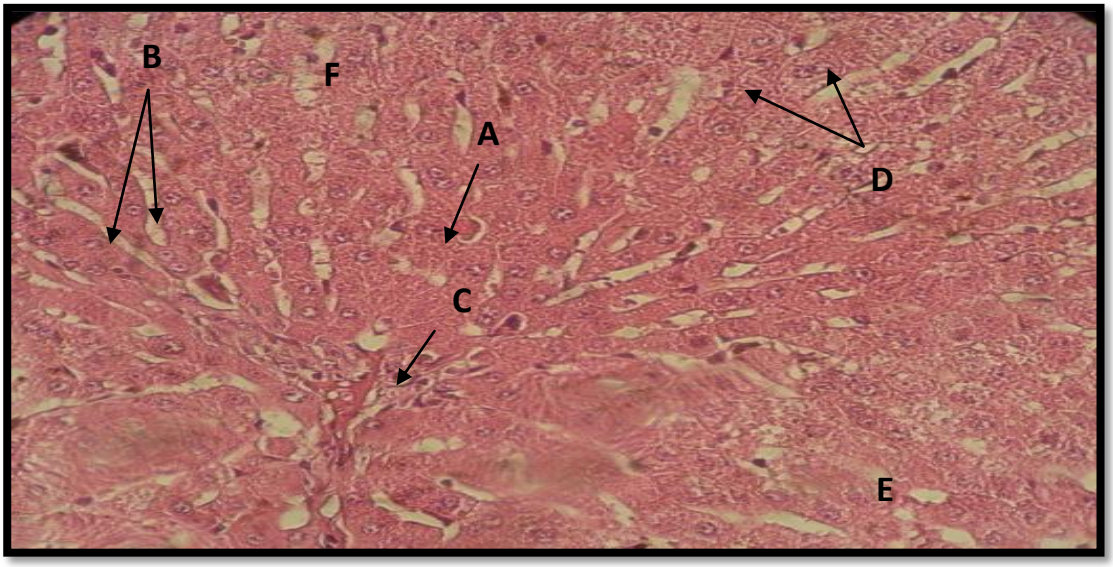

Fig. (5): fetal liver at 14 day of gestation which shows the A- Hepatocyte more prominent nucleus with out lipid droplets inside of cytoplasm. B- Longitudinal hepatic cords. C-prominent kupffer cells with irregular nucleus. D- Elongated liver sinusoid. E- Inner region of liver. F- Outer region of liver. H&E Stain. (100x).

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دراسة نسيجية جنينية- لتطور الكبد في أجنة الأرانب المحلية في مدينة السماوة

باسم عبدالله جاسم **جعفر غازي عباس** **احمد مهدي صالح**
كلية العلوم /جامعة المثنى **كلية الطب البيطري/جامعة القاسم الخضراء** **كلية العلوم /جامعة واسط**

الملخص

استخدم في هذه الدراسة عشرون أرنب محلي بالغ، قسمت هذه الحيوانات إلى اربعة مجاميع كل مجموعة مكونه من أربعة إناث وذكر واحد في مرحلة البلوغ لكل مجموعة هذا التقسيم اعتمد على الفترات الزمنية التي تتابع تطور الكبد خلال المراحل الجنينية المختلفة سميت المجاميع الخمسة من (A,B,C,D) وكل مجموعة تقابل العمر الجنيني المحدد للدراسة أي بعد (6, 8,10,12,14) يوم من عمر الجنين. شملت هذه الدراسة التغيرات النسجية للكبد خلال هذه الفترات الزمنية من عمر الجنين والتي من خلالها نستطيع التعرف على المراحل التطورية التي يمر بها الكبد في المراحل المبكرة من خلال المقاطع النسيجية وباستخدام صبغة الهيماتوكسلين والايوسين. بينت النتائج بان بداية تكوين الكبد تبدأ منذ اليوم السادس من الحمل لوحظ الكبد في بداية التكوين كتركيب برعمي صغير قريب من منطقة تطور القلب في الجنين على شكل كروي. أظهرت النتائج في اليوم الثامن من الحمل بان الكبد أصبح أكثر وضوحا وبدأ بالتمايز ويأخذ مساحه أوسع داخل جسم الجنين.بينت النتائج في اليوم العاشر من عمر الجنين إن اورمات الخلايا الكبدية تلاحظ وبأطوار مختلفة من الانقسامات الخيطية دلالة على تكاثرها عن طريق الانقسام الخيطي. خلال اليوم الثاني عشر من الحمل لوحظ هذه الخلايا تصطف على شكل تراكيب حبليه قصيرة بالاضافة إلى ملاحظه قطرات دهنيه صغيره داخل سايتوبلازم أمهات الخلايا الكبدية. في اليوم الرابع عشر من عمر الجنين لوحظ زيادة الكثافة الخلوية لخلايا الكبد وبدأت بالاصطفاف على شكل تراكيب حبليه طويلة واختفاء القطرات الدهنيه من سايتوبلازم أمهات الخلايا الكبدية وع وجود أعداد كبيره من خلايا الدم الحاوية على نواة حقيقية فظلا عن إن المنطقة المحيطيه للكبد تكون أكثر عتمة من المنطقة المركزية للكبد.